PATENT
Attorney Docket No. 272478US0XPCT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of)
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SEVE, M. and FAVIER, A.)
) Group Art Unit:
Serial No.: 10/535,395)
) Examiner: EWOLDT, Gerald R.
Filed: 04/10/2006)
)

For: PROTEIN SPECIFIC TO PANCREATIC BETA CELLS IN ISLETS OF LANGERHANS AND APPLICATIONS THEROF

Declaration pursuant to 37 C.F.R. § 1.132

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

I, Michel SEVE, do hereby declare and state the following:

1. That I am a Pharmacy Doctor (1995, University of Grenoble, France), and that I received a Master's degree in Structural and Functional Biology (1996, University of Grenoble, France) and a PhD in Structural and Functional Biology (1999, University of Grenoble, France). Since 2005, I have been an hospital biologist, at the head of the Center for Innovation in Biology (University Hospital of Grenoble, France), and I am also the Director of the Scientific Board of Mellitech SAS, which is a biotech focused on developing new therapeutic and diagnostic approaches to diabetes. Enclosed, please find a copy of my *curriculum vitae* and a list of scientific publications, which clearly indicate my expertise in the field of zinc transporters.

2. I am one of the inventors of the above-captioned patent application and therefore I am very familiar with the subject application. I have read and understood the latest Official Action issued by the U.S. Patent and Trademark Office on 04/24/2008. It is my understanding that Claims 39 to 48 were rejected under 35 U.S.C. § 101, as lacking patentable utility. In rendering this rejection, the Examiner asserts that neither the claims nor the specification disclose any reason for the detection of autoantibodies to ZnT-8 or fragments thereof.

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3. In order to address the issue of utility concerning the detection of autoantibodies directed ZnT-8 or the disclosed fragments thereof, I would like to recall some points concerning the present application, and concerning the knowledge of the skilled artisan in the field of diabetes at the date of filing of the present application:

4. **Disclosure of the present application**

The present application describes ZnT-8 as a beta-cell specific protein, which can consequently be used as a new marker for beta cell selective sorting and counting. The fact that ZnT-8 is specifically expressed in the beta cells of the pancreatic islets of Langerhans is disclosed in the present application, at least in the very first paragraph and page 6, lines 24-27.

The claims have been amended to reflect this, by reciting that the autoantibodies which are detected are specific for the beta cells of the pancreatic islets of Langherhans.

Besides, I respectfully note that contrary to the Examiner's assertion, the word "autoantibody" appears in the specification, at least at page 20, third paragraph.

5. **Since many years, the skilled artisan has known of the link between beta cell mass, autoimmunity and type 1 diabetes**

It is clearly known since years that Type 1 diabetes is an autoimmune disease resulting from specific destruction of the insulin-producing beta cells of the Langerhans islets of the pancreas (Cell 85:291–297 (1996), copy of which is enclosed). Two phases can be distinguished: insulitis, when a mixed population of leukocytes invades the islets; and diabetes, when most beta cells have been killed off, and there is no longer sufficient insulin production to regulate blood glucose levels, resulting in hyperglycaemia.

It has been demonstrated that physiological destruction of beta cells is a crucial event at disease outset, initiating autoimmunity against these cells (Nature 414:792-798 (2001), copy of which is enclosed).

6. **At the date of filing of the present application, the skilled artisan knew that auto-antibodies directed against beta-cell antigens could be used as markers of type 1 diabetes**

I would like to draw the Examiner's attention towards two publications which more precisely reflect the general knowledge of diabetologists at the time when the present application was filed.

The first publication is a commentary, entitled "*Autoimmunity and Diabetes*", published in 1999 in the *Journal of Clinical Endocrinology and Metabolism* (Kukreja and Maclaren, 1999, copy of which is enclosed). In this article, the authors have reviewed what was known in 1999 about type 1 diabetes, also called immune-mediated diabetes (IMD). Their review encompasses several aspects of IMD, such

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as genetics, pathogenesis and future prospects regarding the diagnosis and treatment of IMD.

As explained by Kukreja and Maclaren (see page 4373, right column, lines 4-21 and 34-46), IMD results from the destruction of beta cells of the islets of Langerhans, which is a chronic process. At the time of clinical diagnosis of IMD, about 80% of the beta cells have been destroyed, but autoantibodies to beta cells are detectable long before a person develops diabetes. Most importantly, the progression of the disease is quite variable after the onset of islet cell autoimmunity, since some patients rapidly progress to clinical diabetes, while others remain in a non-progressive state. It appears that the nature, intensity, and antigenic spreading of the reactivity of these autoantibodies distinguish individuals who develop diabetes from those who do not. Indeed, antigenic/epitope spreading of the autoantibody responses is one important marker of impending progression, because those with a single autoantibody progress slowly, whereas those with autoantibodies to multiple antigens most often progress rapidly. First discovered with respect to ICA plus insulin autoantibodies, the principle extends to all antibody markers. Diabetes risk and time to diabetes in relatives of patients, thus, directly correlates with the number of different autoantibodies present.

At the end of this review, the authors anticipated that novel important target antigens for T cells that attack the islets of Langerhans would be discovered, as well as appropriate markers to detect autoimmune response of type 1 diabetes in the general population (page 4377, left column, line 26-38).

The second article is a review article published in 2001, entitled "*Prediction and Diagnosis of Type-1 Diabetes Using β-cell Autoantibodies*" (Batstra *et al.*, *Clinical Laboratory*, 2001). In this article, the authors explain that early diagnosis of diabetes, or prognosis before the clinical manifestations become detectable, is important for avoiding macro- and micro-vascular complications due to the deterioration of metabolic control (see at least in the abstract). Batstra *et al.* also mention that discrimination between type 1 and type 2 diabetes is important for determining the most appropriate treatment, and that this can be performed by detecting autoantibodies targeting beta cells of the islets of Langerhans (see page 499, left column, 1st paragraph).

Batstra *et al.* define the "secondary prediction" as a prediction of type 1 diabetes mellitus based on the detection of β-cell-directed autoantibodies in individuals in the prediabetic phase (see page 500, left column). It appears that this prediction is more efficient when combining several antibody tests, especially because of epitope spreading (see page 501, right column, before-last paragraph, and the paragraph bridging pages 501 and 502).

7. **At the date of filing of the present application, diagnostic assays based on the detection of autoantibodies directed against beta-cell antigens were intensively developed**

The diagnostic assay standardization program reported in Diabetes 50:1749-54 (2001) also shows that routine diagnosis of type 1 diabetes based on beta cell-specific auto-antibodies was being implemented before the date of our patent filing.

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8. **At the date of filing of the present application, the skilled artisan, reading the specification, would immediately have recognized the utility of detecting autoantibodies to ZnT-8 and fragments thereof**

Since our patent application clearly describes ZnT-8 as a beta-cell specific protein, and since the skilled artisan in the field of diabetes perfectly knew, at the date of filing of the present application, that auto-antibodies directed against beta-cell specific antigens could be used as markers for the prognosis and diagnosis of type 1 diabetes, the skilled artisan, reading the present specification, would have immediately found useful to detect anti-ZnT-8 autoantibodies, at least in sera from prediabetic and diabetic patients, to confirm that the auto-antibodies could be used as a prognosis/diagnosis marker.

Experiments conducted since the filing of the present application indeed confirmed that detection of auto-antibodies directed against ZnT-8 is useful because it reflects the autoimmune process in a patient enduring type I diabetes. In particular, the amount of autoantibodies directed against ZnT-8 or its fragments reflects the number of beta cells still functional, *i.e.*, beta cell mass, and hence provides important information about the diabetes state.

As a conclusion, I am truly convinced that the claimed method is supported by a substantial asserted utility, because the skilled artisan, at the date of filing, would have found valuable reasons for detecting autoantibodies to ZnT-8 and fragments thereof.

9. I further declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine and emprisonment, or both under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Date 26 September 2008 Michel SEVE

Encl.: - CV and list of publications

- Batstra, M. R., Aanstoot, H. J., and Herbrink, P. (2001). Prediction and diagnosis of type 1 diabetes using beta-cell autoantibodies. Clin Lab 47, 497-507.
- Kukreja, A., and Maclaren, N. K. (1999). Autoimmunity and diabetes. J Clin Endocrinol Metab 84, 4371-4378.

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- Mathis, D., Vence, L., and Benoist, C. (2001). beta-Cell death during progression to diabetes. Nature 414, 792-798.
- Peakman, M., Tree, T. I., Endl, J., van Endert, P., Atkinson, M. A., and Roep, B. O. (2001). Characterization of preparations of GAD65, proinsulin, and the islet tyrosine phosphatase IA-2 for use in detection of autoreactive T-cells in type 1 diabetes: report of phase II of the Second International Immunology of Diabetes Society Workshop for Standardization of T-cell assays in type 1 diabetes. Diabetes 50, 1749-1754.
- Tisch, R., and McDevitt, H. (1996). Insulin-dependent diabetes mellitus. Cell 85, 291-297.

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Curriculum vitae

I. Education and Diplomas

- **Computing Certificate, University of Grenoble, France** *(Jun 1993)*
- **Pharmacy Doctor,** Faculty of pharmacy, University of Grenoble - France *(Feb 1995)*
- **Master of advanced studies in Structural and Functional Biology** *(Jul 1996)*
- **PhD in Structural and Functional Biology** University of Grenoble, France *(Dec* 1999)
- **Accreditation to supervise research** *(Oct 2003)*

II. Postgraduate positions (*italic: current positions*)

- **Research Assistant,** Liverpool, UK *(2000-2001)*
- **Hospital Assistant,** University Hospital of Grenoble *(2001-2005)*
- **Professor Assistant,** Faculty of Pharmacy, University of Grenoble *(Oct 2001 -)*
- **Hospital Biologist *(Praticien Hospitalier)***, University Hospital of Grenoble *(Aug 2005 –)*
  Head of the Center for Innovation in Biology
- **Professor**, Faculty of Pharmacy, University of Grenoble *(Sept 2008-)*

III. Membership of scientific societies

2000-2001: Member of BSCB (*British Society for Cell Biology*). London, UK
2001-: Member of SFBBM (*French society for Biochemistry and molecular biology*), Paris, France.
2003-: Member of SFERETE (*French Society for Research and Study of Essential Trace Elements*), Grenoble, France.
2003-: Member of EFB (*European Federation of Biotechnology*). Frankfurt, Germany.
2004-: Member of SFBC (*French Society of Clinical Biology*) France.

IV. Pedagogical and collective responsibilities

2003-2004: Member of the executive committee of IMBG (Institute of Metals in Biology of Grenoble).
2002- : Teaching and Pedagogical Responsibilities
- Courses in Biotechnologies (192 hours/year)
- Responsible for a Master of Biotechnology

2007-: Member of the direction board of Grenoble School of Pharmacy (University J. Fourier)
2008- : Head of the teaching department "Molecular bases of pathologies and treatments (biochemistry, cellular biology, genetic, Biotechnologies, nutrition) at the Pharmacy School (university J. Fourier)
2008-: In charge of the project "School of Biotechnologies" for Grenoble university
Reviewer for Neuroscience Letters, Diabetologia, Proteome Science, Lung Cancer, Medical Principles and Practice, Journal of Trace Elements in Medicine and Biology.

A. Other responsibilities

Director of the scientific advisory board of the MELLITECH SAS Biotech company (www.mellitech.com).

Publications and communications

Peer-reviewed Publications

1- "The Human Immunodeficiency Virus-1 Tat Protein Increases Cell Proliferation, Alters Sensitivity to Zinc Chelator-Induced Apoptosis, and Changes Sp1 DNA Binding in HeLa Cells"
Seve M., Favier A., Osman M., Hernandez D., Vaitaitis G., Flores N.C., McCord J.M., Flores S.C.
Arch Biochem Biophys 1999 Jan 15;361(2):165-172

2- "Differential remodeling of the HIV-1 Nucleosome upon transcription activators- and SWI/SNF complex-binding"
Angelov D., Charal M., Seve M., Côté J., Khochbin S., Dimitrov S. *J Mol Biol* 2000 Sep 15; 302 (2): 315-326

3- "FGF-2 stimulation of $p42/44^{MAPK}$ phosphorylation and IκB degradation is regulated by heparan sulfate/heparin in rat mammary fibroblasts"
Delehedde M., Seve M., Sergeant N., Wartelle I., Lyon M., Rudland P.S., Fernig D.G. *J Biol Chem.* 2000 Oct 27; 275(43): 33905-33910

4- "The human immunodeficiency virus-1 tat protein impairs selenoglutathione peroxidase expression and activity by a mechanism independent of cellular selenium uptake: consequences on cellular resistance to UV-A radiation"
Richard M.J. Guiraud P., Didier C., Seve M., Flores S., Favier A. *Arch Biochem Biophys* 2001 Fev 15;386(2):213-220

5- "Role of cellular zinc in programmed cell death: temporal relationship between zinc depletion, activation of caspases and cleavage of Sp family transcription factors"
Chimienti F., Seve M., Richard S., Mathieu J., Favier A.
Biochem Pharmacol 2001 Jul 1; 62(1): 51-62

6- "Zinc resistance impairs sensitivity to oxidative stress in HeLa cells: protection through metallothioneins expression"
Chimienti F., Jourdan E., Favier A, Seve M.
Free Rad Biol Med 2001 Nov 15; 31(10): 1179-1190

7- "Rôle du zinc intracellulaire dans la mort cellulaire programmée"
Seve M., Chimienti F., Favier A.
Pathol Biol 2002 Apr; 50(3): 212-221

8- "Zinc homeostasis-regulating proteins: new drug targets for triggering cell fate"
Chimienti F., Aouffen M., Favier A., Seve M.
Current Drug targets 2003; 4(4): 323-38

9- "Differential regulation of zinc efflux transporters ZnT-1, ZnT-5 and ZnT-7 gene expression by zinc levels: a real-time RT-PCR study"
Devergnas S., Chimienti F., Naud N., Peinnequin A., Coquerel Y., Chantegrel J., Favier A., Seve M.
Biochem Pharmacol 2004; 68(4): 699-709

10- "In silico identification and expression of SLC30 family genes: an expressed sequence tag data mining strategy for the characterization of zinc transporters' tissue expression".
Seve M., Chimienti F., Devergnas S., Favier A.
BMC Genomics. 2004; 5(1): 32.

11- "Identification and cloning of a beta-cell-specific zinc transporter, ZnT-8, localized into insulin secretory granules".
Chimienti F., Devergnas S., Favier A., Seve M.
Diabetes. 2004; 53(9): 2330-7.

12- "ZnT-8, a beta-cell-specific zinc transporter".
Chimienti F, Favier A, Seve M.
Biometals. 2005; 18(4): 313-7.

13- "Resveratrol enhances UVA-induced DNA damage in HaCaT human keratinocytes".
Seve M., Chimienti F., Devergnas S., Aouffen M., Douki T., Chantegrel J., Cadet J., Favier A.
Medicinal chemistry 2005;1(6): 629-634

14- "In vivo expression and functional characterization of the zinc transporter ZnT8 in glucose-induced insulin secretion"
Chimienti F, Devergnas S, Pattou F, Schuit F, Garcia-Cuenca R, Vandewalle B, Kerr-Conte J, Van Lommel L, Grunwald D, Favier A, Seve M.
J Cell Sci. 2006 Oct 15;119(Pt 20):4199-206.

15- Peptides OFFGEL electrophoresis: a suitable pre-analytical step for complex eukaryotic samples fractionation compatible with quantitative iTRAQ labeling.
Chenau J, Michelland S, Sidibe J, Seve M.
Proteome Sci. 2008 Feb 26;6(1):9

Conferences proceedings

1- "Intracellular zinc chelation induces apoptosis, caspases activation and transcription factors degradation in Jurkat and HeLa cells"
Seve M., Chimienti F., Richard S., Mathieu J., Favier A. pp 1003-. Trace elements in man and animal 10. Edited by Roussel A.M., Anderson R.A. and Favier A.E. Publié par *Kluwer Academic / Plenum Publishers*, New York, Avril 2000

2- "The zinc transporter ZnT-8 enhances insulin secretion in INS-1E cells"
Chimienti F, Devergnas S, Girod-Roux PM, Favier A, Seve M. *Diabetologia* 2005; 48: A170-A170 459 Suppl. 1

3- "Etude du sécrétome de cellules du cancer du poumon non à petites cellules"
Chenau J, Mondello N, Favrot M, Seve M. *Bulletin du Cancer* 2006; 93: S99

4- "Mise en évidence de marqueurs du cancer du poumon non à petites cellules dans le plasma par la technique de Seldi-Tof (Surface Enhanced laser desorption ionisation-time of flight)"
Michelland S, Godart F, Amblard C, de Fraipont F, Moro-Sibilot D, Garin J, **Seve M**, Favrot MC. *Bulletin du Cancer* 2006; 93: S99
5- "Analyse protéomique des protéines secrétées par des cellules du cancer du poumon non à petites cellules: influence de P53"
Chenau J, Favrot M, **Seve M.** *Bulletin du cancer* 2007, 94: S38
6- "Recherche de biomarqueurs du cancer du poumon non à petites cellules par analyse de profiles d'expression protéique"
Michelland S, Amblard C, De Fraipont F, Moro-Sibilot D, Garin J, **Seve M**, Favrot MC. *Bulletin du cancer* 2007, 94: S38
7- "Stratégie de préfractionnement des échantillons plasmatiques pour la recherche de marqueurs en cancérologie "
Sidibé J, Michelland S, Chenau J, **Seve M**. *Bulletin du cancer* 2008, 95: S19
8- "Peptides Offgel electrophoresis : a suitable pre-analytical step for complex eukaryotic samples fractionation compatible with iTRAQ labeling "
Chenau J, Michelland S, Sidibé J, **Seve M**. *Bulletin du cancer* 2008, 95: S19
9- "Analyse protéomique différentielle des protéines secrétées par des cellules du cancer du poumon non à petites cellules en fonction du statut p53 "
Chenau J, Michelland S, Coll JL, **Seve M** *Bulletin du cancer* 2008, 95: S19
10- "Design of a ligand-fishing method for the characterizaton of proteins associated to DNA lesions generated by cisplatin "
Bounaix Morand Du Puch C, Chantegrel-Gracia J, Favier A, **Seve M**, Sauvaigo S, Breton J. *Bulletin du cancer* 2009, 95: S19

Patent

1- "Protein specific to pancreatic beta cells in islets of Langerhans and applications thereof"
Seve M. et Favier A. (inventors)
Applicant: J. Fourier University and CEA
18 november 2003: WO 2004/046355 A2

Other publications

1- "Amylose de la Bêta-2 microglobuline. Caractérisation de l'épitope d'un anticorps monoclonal. Etude préliminaire de la protéine bêta-2 microglobuline et de l'épitope par dichroïsme circulaire"
Seve M.
Mémoire de thèse de pharmacie, *27 février 1995*
2- "Etude des conditions oxydo-réductrices et de la déplétion en zinc sur le facteur de transcription Sp1"
Seve M.
Mémoire de DEA, *juillet 1996*
3- "Les peroxydes lipidique et protéique".
Seve M.
Atelier N°8: Radicaux libres.
XVI^{ème} Journées Nationales de Biologie - SFBC, Lyon, France, *6-7 juin 1997*
4- "Les facteurs de transcription de la famille Sp: Structure et fonctionnalité"
Seve M.
Mémoire de thèse (Biologie structurale et fonctionnelle), *21 Décembre 1999*
5- "Le transport et l'homéostasie du zinc cellulaire"
Seve M., Chimienti F., Jourdan E., Favier A.
Regard sur la Biochimie 2001; 3
6- "Métabolisme du zinc"
Seve M., Favier A.
Chapitre Endocrinologie/Nutrition 10-359-D-10 *in* Encyclopédie Médico-chirurgicale 2002, 16p, Editions Scientifiques et Médicales Elsevier SAS, Paris.
7- « Zinc et insuline »
Seve M.
Feuille rouge. Brèves du CEA
8- "Mécanismes cellulaires du transport du zinc chez les mammifères"
Garcia-Cuenca R., Chimienti F., **Seve M.**, Favier A.
Regard sur la Biochimie 2005
9- "Présent et futur de la Protéomique Clinique - Bilan d'activité du Groupe de travail « Protéomique Clinique » de la SFBC 2004-2006"
Lehmann S, Dupuy A, Peoc'h K, Roche S, Baudin B, Quillard M, Berger F, Briand G, Chwetzoff S, Dine G, Gonzalo P, Dastugue B, **Seve M**, Siest G et Beaudeux JL
Ann Biol Clin 2007 Sep-Oct;65(5):463-71
10- "Le Sécrétome: Définitions et intérêt bio-médical"
Chenau J., Michelland S., **Seve M**.
La revue de Médecine Interne, 2008
11- « Les Marqueurs Biologiques, définitions et concept »
Seve M., Favier A.
In « Marqueurs en Biochimie Clinique », Lavoisier Ed., 2008 *in press*